COMPARISON OF THREE DIFFERENT TECHNIQUES FOR EXTRACTION OF VOLATILES FROM PISTACHIO NUTS

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Abstract

Pistachio nut (*Pistacia vera* L.) is one of the popular tree nuts of the world and is widely cultivated in saline, dry and hot areas of the Middle East, Mediterranean countries and United States. Iran, USA, Syria and Turkey are the main producer countries of this product. Pistachio nut is widely consumed as a raw or roasted ingredient of many desserts, ice creams, cakes, pastry and in the production of some sausages. The aim of the present study was to determine the appropriate technique for extraction of volatiles from pistachio nuts. Uzun variety which is cultivated in the South-East Region of Turkey was used as the material of the study. Volatiles were extracted with three different extraction techniques – Liquid-Liquid Solvent Extraction (LLSE) at 85±2 °Cfor 6 h by using ethanol and hexane as solvents, Headspace extraction (HS) at 100°C for 1 h, and Solid Phase Micro Extraction (SPME) at 100 °C for 1 h by using DVB/Car/PDMS fiber. Obtained extracts were analyzed by Gas Chromatography/Mass Selective detector (GC/MS). The total number of volatiles extracted by LLSE, HS and SPME techniques were 34, 41, 25, respectively; and the percentage of volatiles identified by these techniques were found to be 67.36%, 72.04% , 92.91%, respectively. Since more than 90% of volatiles could be identified by SPME which also showed high repeatability, it could be recommended as the most appropriate technique for extraction of volatiles from pistachio nuts.

Keywords: pistachio nut, extraction of volatile components, GC/MS analysis.

Introduction

Pistachio nut (*Pistacia vera* L.) is one of the popular tree nuts of the world and is widely cultivated in saline, dry and hot areas of the Middle East, Mediterranean countries and United States (Maskan, Karatas, 1999; Kashani-Nejad et al., 2003). According to the FAOSTAT Iran, USA and Turkey are the main producer countries of pistachio nuts (Anon, 2014a). One of the major cultivar of pistachio nuts grown in Turkey is Uzun and it is mainly preferred for the production of baklava and nut paste because of its special green kernel colour, flavour and texture (Balta, 2002; Gamlı, Hayoglu, 2007).

Different kind of extraction techniques have been studied to determine the volatile compound of different types of nuts such as solvent extraction technique for chestnuts (Morini, Maga, 1995), almonds (Cantalejo, 1997; Vazquez-Araujo et al., 2008), peanuts (El-Kayati et al., 1998; Ku et al., 1998), hazelnuts (Kiefl, Schieberle, 2013) headspace (HS) technique for peanuts (Young, Hovis, 1990; Braddock et al. 1995; Burroni et al., 1997; Ku et al. 1998, Alasalvar et al., 2003); and solid phase micro extraction (SPME) technique for peanuts (Abegaz et al., 2004; Krist et al., 2004. Although the flavour of pistachio nuts has wide appeal, there is not enough study about this topic. Soliman et al (1981) studied the volatiles of roasted pistachio nuts by using vacuum carbon dioxide distillation of acetone extracts and identified the volatiles as pyrazines, pyrrols, aldehydes and some others by using GC/MS. In another study conducted by Kendirci and Altug (2011) the volatile compounds of different varieties of fresh pistachio nuts were extracted by using SPME-GC/MS and it was found that volatiles of fresh pistachio nuts were mainly composed of terpenes like α-pinene, α-terpinolene, limonene, β-myrcene. In another study where volatiles of roasted pistachio nut were analysed, pyrazines, aldehydes, acids and some other compounds were detected (Acena et al. 2011).

In this study, the volatile components of Uzun variety of pistachio nuts grown in Turkey were extracted using three different extraction methods to determine the appropriate technique. For this purpose, Liquid-Liquid Solvent Extraction (LLSE), Headspace extraction (HS) and Solid Phase Micro Extraction (SPME) was applied to the pistachio nuts. Obtained extracts were analyzed by Gas Chromatography equipped with Mass Selective Detectorr (GC/MS).

Materials and Methods

Materials

Uzun variety of pistachio nuts (*Pistacia vera L.*) were obtained from Pistachio Nut Research Institute in Gaziantep, Turkey at the beginning of the harvest season in September 2005.

Liquid Liquid Solvent Extraction (LLSE)

The method described by Heath and Reineccius (1986) was applied to the samples as a LLSE technique. For this purpose, 40 g of ground pistachio nuts were transferred to the sample tube of LLSE apparatus with the help of 100 mL of ethanol; while 125 mL of hexane was in the collector conical flask (Figure 1).

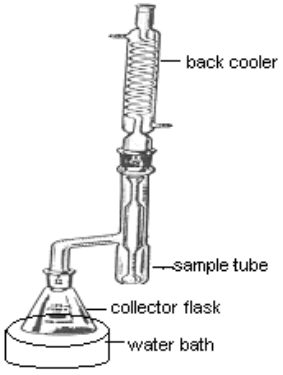

Figure 1. LLSE apparatus

Conical flask was placed in to the water bath at 85±2 °C and extraction was continued for 6 h. Hexane extract was concentrated to 10 mL at room temperature by the help of nitrogen gas. 5 µL of extract was injected in the GC/MS (Hewlett Packard, USA).

Headspace Extraction (HS)

HS extraction was performed by modifying the methods suggested by Young, Hovis (1990), Burroni et al. (1997) and Ku et al. (1998). For this purpose 8 g of ground sample was weighed into a screw top amber vial with black viton septa which was placed into a block heater at 100 °C. After 60 min, 1 mL of headspace was injected to the GC/MS device.

Solid Phase Micro extraction (SPME)

SPME procedure was performed by using a manual SPME device and extractions were carried out by using a 50 / 30 µm DVB/Carboxen/PDMS stable-Flex fiber as suggested in Abegaz et al. (2004) and Krist et al. (2004). 8 grams of dehulled and groundsample was weighed into a screw top amber vial with black viton septa which was placed into a block. SPME fibre was immersed to the headspace of the vial and the volatiles were collected for 60 min at 100 ºC. After sampling, the SPME fiber was removed from the vial and introduced onto the injection port of GC/MS.

GC/MS Analysis

The volatiles were separated using HP-5MS 5% phenylmethylcyloxane column (30.0 m×0.25 mm× 0.25 µm film thickness) that was attached to a Hewlett Packard Model HP-6890 gas chromatograph equipped with a HP 5973 Mass Selective detector. The operating conditions of the GC were as follows: injector temperature of 250 °C, splitless mode, carrier gas of helium (with an inlet flow rate of 1 mL min^{-1}). The temperature gradient used began at 60 °C for 1 min, then was raised to 260 °C at 5 °C min^{-1} and held at this temperature for 45 min. Mass spectra were generated at 70 eV. The mass selective detector was scanned from 30 to 350 at 1 scan s^{-1}. Identification of the volatile compounds was achieved by comparing retention times with those in the MS library (NIST and WILEY).

Interpretation of the Results

The analyses were applied as triplicate for each technique, and the standard deviations from the mean values were calculated and shown on tables.

Results and Discussion

The chromatographs of the volatiles extracted from the pistachio nuts by using LLSE, HS and SPME techniques are shown on Figures 1–3; list of the volatile compounds are given in Tables 1–3.

As it can be seen from Table 1 and Table 4, 34 volatile compounds were extracted from pistachio nuts using LLSE technique. 32.62% of the total peak area of the volatiles could not be identified, while 35.41% them were determined as 9-octadecanoic acid isomers having a fatty character (Anon 2014b).The percentage of α-pinene which is one of the major volatiles of the pistachio nuts (Kendirci, Altug, 2011) was detected to cover only 0.16% of the total peak area. The repeatability of the technique was determined to be low since the standard deviations of the results were high from Table 1.

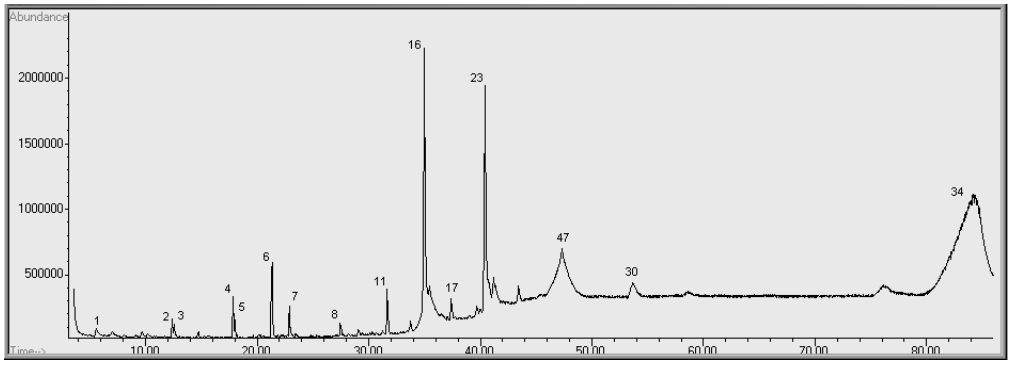

Figure 1. GC/MS chromatograph of the volatiles extracted by using LLSE technique

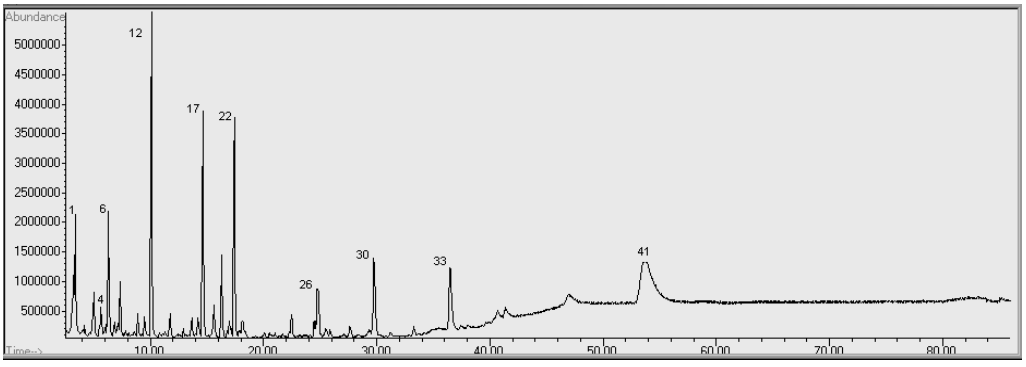

Figure 2. GC/MS chromatograph of the volatiles extracted by using HS technique

Table 1

The volatile compounds extracted by using LLSE technique

Peak	t_R (min)	Volatile compound	Area %
1	5.60	α-pinene	0.16±0.28
2	12.4	1-dodecene	0.57±0.49
3	12.58	dodecane	0.47±0.41
4	17.87	1-tetradecene	1.30±0.97
5	18.02	tetradecane	0.37±0.53
6	21.34	2,6-bis(1,1-dimethylethyl)-4-phenol	1.81±0.97
7	22.89	1-hexadecene	1.13±1.07
8	27.44	5-octadecene (E)	0.59±0.57
9	29.07	hexamethyl pyranonyndane	0.04±0.07
10	31.19	Unidentified	0.04±0.08
11	31.63	Hexadecanoic acid ethylester (ethyl palmitate)	1.10±0.98
12	32.28	n-hexadecanoic acid	0.62±1.07
13	32.85	9-octadecanoic acid (Z), isomer	1.15±1.07
14	33.40	5-eicosene	0.35±0.61
15	33.74	9-octadecanoic acid (Z), isomer	0.63±0.34
16	34.96	ethyl oleate	10.17±7.50
17	35.70	9-octadecanoic acid (Z), isomer	19.46±26.69
18	36.51	9-octadecanoic acid (Z), isomer	1.13±0.78
19	37.40	Unidentified	1.26±0.60
20	39.01	9-octadecanoic acid (Z), isomer	0.07±0.12
21	39.72	9-octadecenal	0.60±0.98
22	40.00	Unidentified	0.14±0.24
23	40.41	9,17-octadecadienal (Z)	13.74±8.86
24	40.8	Unidentified	0.52±0.91
25	41.17	Unidentified	2.91±3.12
26	41.40	1,12-tridecadiene	1.43±2.47
27	43.45	Unidentified	1.38±0.70
28	45.42	9-octadecenoic acid (Z) , 3-hydroxypropyl ester	2.47±4.28
29	47.31	9-octadecanoic acid (Z), isomer	6.55±7.36
30	53.73	9-octadecanoic acid (Z), isomer	3.95±2.08
31	58.77	Unidentified	0.27±4.28
32	61.05	Unidentified	4.15±7.18
33	76.08	Unidentified	2.97±2.38
34	84.28	Unidentified	15.03±26.03

Table 2

The volatile compounds extracted by using HS technique

Peak	t_R (min)	Volatile compound	Area %
1	3.36	hexanal	8.20±0.67
2	4.20	2-ethyl-3-vinyloxirane	0.18±0.25
3	5.04	heptanal	2.32±0.28
4	5.66	α-pinene	0.73±1.04
5	6.05	Unidentified	0.24±0.34
6	6.35	2-heptenal, (E)	4.56±0.31
7	6.85	heptanol	0.32±0.45
8	7.14	1-octen-3-ol	9.29±0.41
9	7.39	octanal	2.54±0.38
10	8.96	octenal	0.89±0.04
11	9.58	penthylcyclopropane	0.92±0.12
12	10.15	nonanal	9.60±0.95
13	11.82	2-nonenal	1.05±0.07
14	12.93	decanal	0.17±0.24
15	13.68	Unidentified	0.31±0.43
16	14.29	Unidentified	0.98±0.04
17	14.71	2-decenal (E)	7.91±0.07
18	15.70	2,4-decadienal (E,E)	1.58±0.16
19	16.39	2,4-decadienal	3.12±0.12
20	16.82	Unidentified	0.12±0.17
21	17.00	Unidentified	0.51±0.72
22	17.50	2-octenal	7.96±0.30
23	18.13	Unidentified	1.00±1.42
24	22.55	8-hexadecane (Z)	1.16±0.08
25	24.47	Unidentified	0.23±0.33
26	24.85	8-heptadecene	2.88±0.43
27	25.51	1-cloro tetradecane	0.27±0.39
28	27.62	7-hexadecenal (Z)	0.30±0.42
29	29.35	Unidentified	0.26±0.37
30	29.82	9-oxabicyclo[6.1.0]nonane	4.83±0.21
31	33.33	Unidentified	0.55±0.15
32	35.58	9-octadecanoic acid (Z)	3.78±5.34
33	36.54	Unidentified	6.87±0.05
34	37.45	E,E-10,12-hexadecadiene-1-ol acetate	0.12±0.17
35	39.83	9-octadecanoic acid (Z), isomer	0.41±0.59
36	40.51	9-octadecanoic acid (Z), isomer	0.97±1.38
37	40.76	9-octadecanoic acid (Z), isomer	2.49±1.31
38	41.46	9-octadecanoic acid (Z), isomer	2.56±1.91
39	46.95	Unidentified	1.28±1.81
40	47.33	Unidentified	2.02±2.85
41	53.94	Unidentified	13.53±4.38

41 volatiles could be extracted from the pistachio nuts and the repeatability of HS technique was higher in comparison with LLSE technique (standard deviations are lower than LLSE) (Table 2). On the other hand, 27.90% of the total extracted volatile compounds could not be identified and the percentage of α-pinene was found to be only 0.16% (Table 4).

Table 3 shows that 25 volatile compounds were extracted from samples by using SPME technique.

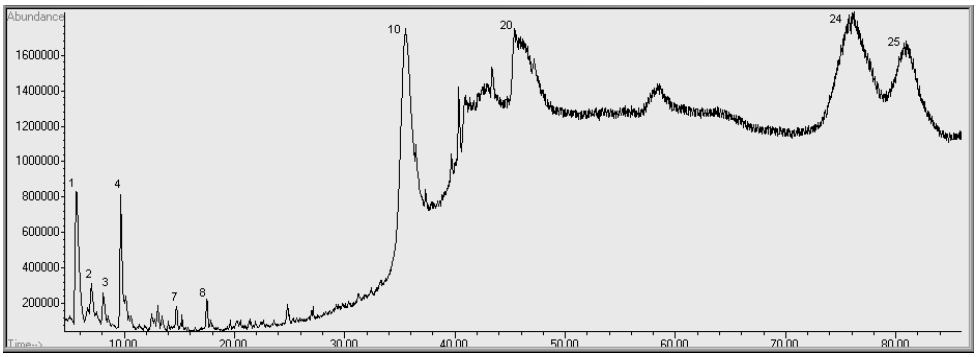

Figure 3. GC/MS chromatograph of the volatiles extracted by using SPME technique

Table 3

The volatile compounds extracted by using SPME technique

Peak	t_R (min)	Volatile compound	Area %
1	5.64	α-pinene	10.23±8.85
2	7.01	β-myrcene	1.80±1.32
3	8.09	limonene	1.41±1.25
4	9.67	α-terpinolene	6.38±5.90
5	10.16	o-isoprophenyltoluene	0.16±0.22
6	13.04	azulene	0.14±0.20
7	14.72	2-decenal	0.61±0.40
8	17.48	2-octenal	0.73±0.56
9	24.78	8-heptadecen	0.15±0.21
10	35.43	9-octadecanoic acid (Z), isomer	31.09±17.36
11	36.44	9-octadecanoic acid (Z), isomer	3.07±0.05
12	37.33	9-octadecanoic acid (Z), isomer	0.24±0.33
13	39.68	9-octadecanoic acid (Z), isomer	0.14±0.20
14	40.33	Unidentified	2.10±1.69
15	40.67	9-octadecanoic acid (Z), isomer	2.85±4.03
16	40.91	Unidentified	0.69±0.98
17	41.29	Unidentified	1.32±0.98
18	41.45	9-octadecanoic acid (Z), isomer	0.56±0.78
19	43.36	Unidentified	1.44±0.87
20	45.33	9-octadecanoic acid (Z), isomer	4.47±1.24
21	46.00	9-octadecanoic acid (Z), isomer	2.43±3.44
22	47.20	9-oxabicyclo[6.1.0]nonane	1.53±2.17
23	58.57	Unidentified	1.54±2.17
24	76.13	9-octadecanoic acid (Z), isomer	15.29±21.63
25	80.97	9-octadecanoic acid (Z), isomer	9.64±13.63

Although the total number of the volatiles extracted by SPME were lower in comparison with the other two techniques, the percentage of unidentified volatiles were much lower (7.09%).

Table 4

The names and the proportion of volatile compound groups extracted by using three different extraction techniques (LLSE, HS and SPME)

	LLSE	HS	SPME
TERPENES	0.16 % - α-pinene	0.73% - α-pinene	19.96% - α-pinene - α-terpinolene - β-myrcene - azulene - limonene
ALDEHYDES	14.34% - 9,17-octadecadienal - 9-octadecenal	50.20% - 2,4-decadienal - 2-decenal - 2-heptenal - 2-octenal - 7-hexadecenal - decanal - heptanal - hexanal - nonanal - 2-nonenal - octanal - 2-octenal - 2,4-decadienal	1.34% - 2-decenal- 2-octenal
ACIDS	47.30% - 9-octadecanoic acid - 9-octadecenoic acid -3-hydroxy-propyl ester - ethyl oleate -hexadecanoiacid ethylester - n-hexadecanoic acid	10.21 % - 9-octadecanoic acid isomers	69.78% - 9-octadecanoic acid isomers
OTHERS	8.06 % - 1-dodecene - dodecane - tetradecane - 1-hexadecene - 1-tetradecene - 5-eikosene - 5-oktadecene - 1,12-tridecadiene - 2,6-bis(1,1-dimethylethyl)-4-phenol -,hexamethyl pyranonyndan	19.97 % - 1-cloro tetradecane - 8-heptadecene - 8-hexadecane - 9-oxabicyclo[6.1.0] nonane - 1-octen-3-ol - 2-ethyl-3-vinyloxirane - penthylcyclopropane - E,E-10,12-hexadecadiene-1-ol asetate - heptanol	1.84% - 8-heptadecen - 9-oxabicyclo [6.1.0] nonane - o-isoprophenyl-toluene

Additionally, it was determined that 69.78% of the total peak area of volatiles was composed of 9-octadecanoic acid isomers (Table 4) which gives fatty character (Anon, 2014b); while 7.79% was limonene and α-terpinolene which gives citrus character (Anon, 2014b), 5.64% was α-pinene which gives pine character (Anon, 2014b), 1.80% was β-myrcene which gives must, balsamic and spice characters (Anon 2014b), and 1.34% was 2-decenal and 2-octenal which give green, nut and fatty characters (Anon, 2014b) of pistachio nuts.

Table 4 shows the proportion of volatile compound groups extracted from pistachio nuts by using three different extraction techniques. As it can be seen from the Table 4, among the extraction techniques examined in this study, SPME was the best technique for extracting the terpene groups of the volatiles which are expected to be one of the main volatile groups of pistachio nuts (Kendirci, Altug, 2011) while aldehydes were the main group of HS technique. Because of the high fat content of the pistachios, mainly fatty acids (47.30 %) were determined by LLSE technique (Table 4).

Conclusions

Among the three extraction technique studied in this study, SPME seems to be the best technique for the extraction of the volatile compounds of pistachio nuts. By this technique the major volatiles which make up pistachio nut flavour could be extracted, and the percentage of unidentified compound were detected to be low in comparison with the other tecniques examined in this study

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